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Modulation of Extracellular Levels of 5-HT in the Caudate Putamen of Freely Moving Rats by High Frequency Stimulation of the Subthalamic Nucleus

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Abstract: Electrical high frequency stimulation (HFS) in the subthalamic nucleus (STN) has been shown to have a therapeutic effect in several movement disorders. But, debilitating psychiatric effects like depression and suicidality are occasionally seen and might be caused by the changes in the serotoninergic activity. Previous studies could show that HFS of the STN results in inhibition of the serotonergic neurons originating in the dorsal raphe nucleus. The aim of this study was to characterize the effect of HFS (124 Hz, 0.5 mA) in the STN, on the extracellular levels of serotonin, dopamine and their metabolites HIAA, DOPAC and HVA in the caudate-putamen (CPu) in conscious and freely moving rats. Extracellular levels of the neurotransmitters and their metabolites were quantified using high performance liquid chromatography with electrochemical detection. Under HFS conditions, a significant reduction in the extracellular levels of serotonin was observed. Cessation of HFS showed a recovery back to basal levels. Dopamine levels were not affected, although significant increase of its metabolites DOPAC and HVA were measured. In the case of low frequency stimulation (LFS), levels of serotonin and its metabolite HIAA remained unchanged, while the levels of dopamine metabolites, DOPAC and HVA, showed a significant decline. These results demonstrate evidence for a strong linkage between HFS in the STN and reduction of the levels of serotonin in the caudate-putamen, which is likely responsible for psychiatric side effects seen in Parkinsonian patients who are treated with STN stimulation.

1. INTRODUCTION

Deep brain stimulation of the subthalamic nucleus (STN) has been used successfully as a treatment for alleviating the movement disabilities that are associated with Parkinson's disease [1, 2]. In the case of advanced Parkinson's disease, electrical high frequency stimulation (HFS) at 130 Hz, has been proved to provide cessation of resting tremor, rigidity and hypo- or bradykinesia, and also helps to reduce L-Dopa induced dyskinesia [3, 4]. Recent trials show that STN-HFS is superior to conventional medical management since it lead to a decrease in severity and duration of periods of immobility and dyskinesia. These changes led to an improvement in measurements of activities of daily living and bodily discomfort [5-7].

However, STN-HFS can also be unfortunately associated with debilitating non motor side effects, which include but are not limited to mood alterations and an increased mood alteration, psychosis, impulse control disorders and suicide risk [3, 8]. Systematic reviews in clinical series showed that up to 30% of patients developed depressive symptoms after the onset of STN- HFS [9, 10]. Many of these effects that are seen to arise from high frequency stimulation of the STN (STN-HFS), are thought to arise from reduced functioning of the serotonergic (5-HT) neurons in the brain stem, which is the primary source for the 5-HT innervation to the forebrain. Evidence that STN-HFS affected the 5-HT mechanism has been provided recently, where bilateral stimulation of the STN was shown to inhibit the firing rate of 5HT neurons in the dorsal raphe nucleus (DRN) along with neurochemical studies that showed that the extracellular levels of 5HT in the prefrontal cortex and the hippocampus were significantly affected by STN-HFS [11]. The inhibition was noticeable during HFS and a prolonged inhibition of the 5-HT cell firing was observed [12, 13].

This study investigated the effect of unilateral HFS-STN on the extracellular concentration in the caudate putamen of freely moving rats, using *in vivo* microdialysis. Extracellular 5-HT release was quantified from the ipsilateral caudate putamen (CPu), as it receives dense innervation from the DRN 5-HT system [14, 15]. The effects on the metabolic rate of the monoamines into their metabolites were also studied since changes in the metabolic rate could indicate larger neurochemical changes that need to be further studied and understood.

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2. MATERIALS AND METHODS

2.1. Animals

Male Wistar rats (300-350 grams; Janvier, France) were housed separately under standard lighting conditions (12 h light-dark cycle, lights on 06:00 h - 18:00 h), 22 $^{\circ}$C and 40 % humidity with free access to food and water. All procedures with animals were reviewed and approved by the University of Lübeck and the Ministry for Agriculture, the Environment and Rural Areas, Schleswig-Holstein, Germany, and were conducted in accordance with the NIH guide for the Care and Use of laboratory animals (NIH Guide for the Care and Use of Laboratory Animals, NIH Publication No. 85-23, 1985).

2.2. Implant and Surgery

The target region for electrical stimulation was the subthalamic nucleus (STN) and the microdialysis was carried out from the ipsilateral caudate-putamen (CPu). A custom built double guiding cannula implant was crafted to target these regions. The double guide cannula consisted of a commercially available microdialysis cannula (CMA/11, Carnegie Medicine, Stockholm , Sweden) of which the tubing was replaced and shaped so as to accept the modified stimulation electrode, to which a commercially available microdialysis cannula (AT4.15.iC, AgnTho's, Lidingö, Sweden) was glued by two component epoxy resin under microscopic and micromanipulator control. The first cannula served as a guide for the concentric bipolar stimulation electrode with 250 μm outer diameter (for details see section 2.4), whereas the second cannula served as a guide for the microdialysis probe

Prior to surgery, rats were pre-anesthetized with isoflurane and then, injected with 80 mg/kg ketamine (Ketavet[®], Pfizer) and 1 mg/kg xylazine (Rompun[®], Bayer) intraperitonially. The anesthetised rats were then placed into a stereotaxic frame where body temperature was maintained (37 °C). Once the skin was incised to expose the cranial landmarks, stereotactic coordinates derived from the Paxinos and Watson atlas (Paxinos and Watson, 2007) were used to locate the target regions. The coordinates used were AP_B-3.8, ML_B-2.6 and DV +7.8 for the STN, and AP_B-1.0, ML_B-4.4 and DV +6.0 for the CPu. The implant was fixed in position using skull screws and dental cement. The animals were allowed to recover for 3 days after the surgery before the microdialysis experiments were carried out.

2.3. Microdialysis

After recovery time (Fig. **1**), microdialysis experiments were carried out in conscious freely moving rats as described by Hiller *et al.* [16]. Experiments began with placing the rats in a bowl (Plexiglas, 50 cm diameter). Subsequently, the microdialysis probe with a 4 mm membrane (Agn Tho's, AT4 series, 6 KDa cutoff) and 240 μm diameter was inserted into the corresponding implanted guiding cannula to a depth of DV+6.0 mm from the dural surface. Additionally, a modified bipolar concentric stimulation electrode, with a 45° beveled tip with an internal diameter of 250 μm was inserted to a depth of DV+7.8 from the dural surface (Paxinos and Watson, 2007).

For all experiments, the microdialysis probe was continuously perfused with artificial cerebrospinal fluid (aCSF) with a flow rate of 1.2 μl/min. The aCSF was composed of 125 mM NaCl, 25 mM $NaHCO_3$, 1 mM $MgSO_4$*$7H_2O$, 2 mM $CaCl_2$ *$2H_2O$, 5 mM KCl, 1.25 mM KH_2PO_4 (Merck Chemicals) and 0.1 mM ascorbic acid (Sigma-Aldrich), dissolved in HPLC grade H_2O (J.T. Baker), and the pH was adjusted to 7.4 [17, 18].

The microdialysis probe was perfused with aCSF for 120 minutes before sample collection began. Samples were collected every 20 minutes for 12 consecutive fractions. 24 μl of dialysate was collected into 5 μl 3.3 % perchloric acid (Merck Chemicals). Once the neurotransmitter values stabilized, 3 x 20 min samples were collected to obtain a baseline value followed by 2 x 20 min during stimulation and 6 x 20 min post stimulation (Fig. **1**). All collected samples were stored at -30 °C, and subsequently measured by means of high performance liquid chromatography (HPLC) with electrochemical detection (ED).

2.4. STN Stimulation Protocol

Bipolar platinum–iridium (Pt/Ir) electrodes (GBCBG30, FHC Inc., Maine) were used, with the cathode in the center (diameter 75μm), concentrically surrounded by an anode [19]. The total area of the smaller inner contact was 0.0062 mm^2. For the stimulation, the electrodes were connected to a stimulator (Isostim A320D, WPI, Germany). The stimulation parameters used varied for the STN-HFS (High Frequency Stimulation) group and the STN-LFS (Low Frequency Stimulation) group.

The animals were grouped based on the stimulation parameters, HFS (n=9), LFS (n=16) and non-stimulated control (n=15). For STN-HFS, animals were stimulated with mono-
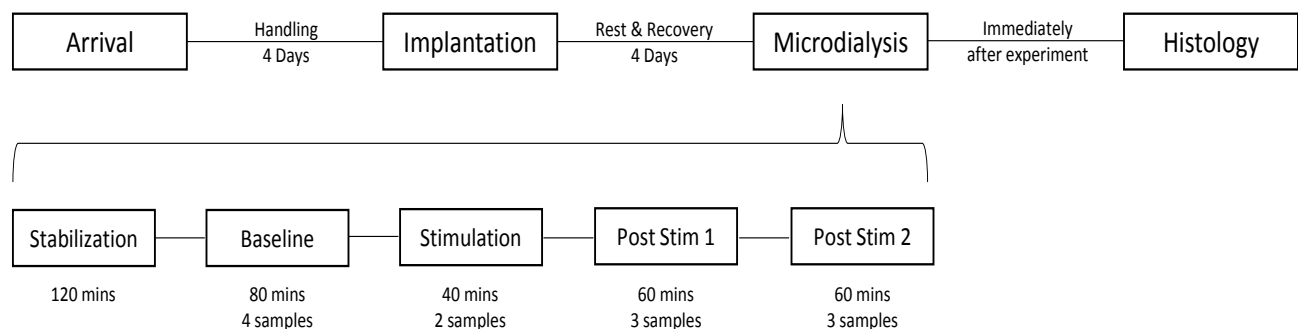

Fig. (1). Experimental layout. Shows grouping of samples and whole schematic for the experiments carried out.

phasic, rectangular pulses at 124 Hz, 0.5 mA constant current, 8 ms pulse width and 60 µs pulse duration. The parameters were chosen on the basis of previous experiments (Hiller *et al.*, 2007). For STN-LFS, animals were stimulated with monophasic, rectangular pulses with frequency of 30 Hz and 0.5 mA constant current. Pulse width was controlled on-line by an oscilloscope (Tektronic 2004B) in parallel with the electrode. In non-stimulated control animals, the electrode was inserted into the guide cannula and connected to the stimulator without stimulation.

2.5. HPLC-ED

The dialysates were analyzed for serotonin (5-HT), 5-hydroxyindoleacetic acid (HIAA), dopamine (DA), 3, 4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) using HPLC-ED. The HPLC system consisted of a C18 column (Eurospher RP 18.5 µm, column size 250 x 4 mm with a pre-column 35 x 4 mm). The isocratic mobile phase (0.15 M sodium acetate buffer, pH 4.0, containing 0.05 mM EDTA, 0.1mM sodium octyl sulphate, and 12% methanol) [20], previously degassed with helium, had a flow rate of 1.0 ml/min (Smartline pump 1050, Knauer, Berlin, Germany) in a temperature-controlled environment (30 $^{\circ}$C). The dialysates were detected electrochemically using a glassy carbon electrode set at a potential of 800 mV relative to an Ag/AgCl reference electrode (Electrochemical Detector CLC 100, Chromsystems GmbH, Munich, Germany). Calibration was carried out daily using standard solutions of the substances we measured.

2.6. Statistical Analysis

Microdialysis data is presented as percentages of the mean of four baseline samples prior to STN stimulation. The effect of HFS on extracellular neurotransmitters and their metabolites was assessed statistically by comparing the normalized values over 4 time periods (baseline, stimulation, post-stimulation 1 and post-stimulation 2) using student's t-tests for pairwise comparisons. Mean neurotransmitter and metabolite values were expressed in nM ± standard error (SEM). Differences between the groups were assessed with one-way analysis of variance (ANOVA) with post hoc analysis for pairwise comparisons, as mentioned.

3. RESULTS

The basal concentrations in all three groups (Control, STN-HFS and STN-LFS) of dopamine (DA) and serotonin (5-HT) and their metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) and 5- hydroxyindoleacetic acid (HIAA) respectively, could be quantified in the nanomolar concentration in the dialysates from the caudate putamen of the rat (Table **1**). The basal levels of the neurotransmitters showed no significant changes between the three groups of animals. To reduce the effect of inter-individual differences of the basal value concentration of the neurotransmitters and their metabolites, the values were normalized and the basal values were set to be 100%.

It was observed that the metabolic rate of 5-HT to HIAA was higher than the metabolic rate of dopamine to its metabolite, HVA, by the ratio of 5-HT: HIAA vs. DA: HVA during the basal time period (Table **2**). When STN-HFS was

carried out, the metabolic rate of 5-HT to HIAA showed a tendency to decrease. In the case of dopamine, the metabolic rate was decreased resulting in an increase in the metabolite concentrations during the stimulation time period. When LFS was carried out, the metabolic rate of 5-HT to HIAA remained constant and no change was observed. However a marked reduction in the dopamine metabolism to its metabolites, DOPAC and HVA was observed (Table **3**).

Table 1. Basal concentrations of neurotransmitters and their metabolites from the rat caudate putamen (CPu). Concentration expressed in nM ± SEM.

Basal Levels			
Dialysate	**Control**	**High Freq. Stim.**	**Low Freq. Stim.**
5-HT	3.03 ± 0.46	2.94 ± 0.26	3.29 ± 0.27
HIAA	24.44 ± 5.93	21.17 ± 3.35	22.32 ± 3.22
DA	15.94 ± 5.01	19.23 ± 2.56	15.68 ± 0.70
DOPAC	37.94 ± 4.97	32.81 ± 5.07	32.51 ± 3.83
HVA	50.55 ± 4.91	50.61 ± 8.84	55.82 ± 4.95

Table 2. The ratio of basal levels of neurotransmitters as measured from the rat caudate putamen. Expressed in Ratio ± SEM.

	Ratio of Neurotransmitter to Metabolite		
	Control	**High Freq. Stim.**	**Low Freq. Stim.**
5-HT: HIAA	0.14 ± 0.02	0.16 ± 0.02	0.16 ± 0.02
DA: HVA	0.35 ± 0.15	0.37 ± 0.07	0.30 ± 0.04
DA: DOPAC	0.37 ± 0.12	0.43 ± 0.06	0.31 ± 0.06

Table 3. The ratio of concentration of neurotransmitters during stimulation as measured from the rat caudate putamen during (A) and after stimulation (B).

(A)

Ratio		
	H.F.S	**L.F.S**
5-HT: HIAA	0.25 ± 0.04	0.21 ± 0.02
DA: HVA	0.39 ± 0.07	0.53 ± 0.12
DA: DOPAC	0.34 ± 0.07	0.48 ± 0.09

(B)

Ratio		
	H.F.S	**L.F.S**
5-HT: HIAA	0.30 ± 0.08	0.21 ± 0.02
DA: HVA	0.39 ± 0.06	0.79 ± 0.16*
DA: DOPAC	0.32 ± 0.05	0.67 ± 0.11

In comparison to non-stimulated sham operated controls, high frequency stimulation of the subthalamic nucleus (STN) for 40 minutes resulted in a delayed but statistically significant decrease in the levels of 5-HT in the ipsilateral caudate putamen (Fig. **2**). This effect persisted and was present for 40 minutes after stimulation was ceased. 140 minutes later during the experiment, the extracellular levels of 5-HT reached near baseline levels again. STN-HFS caused significant decrease in the extracellular levels of HIAA (Table **4**). STN-LFS had no significant effects on extracellular levels of either 5-HT or 5-HIAA.
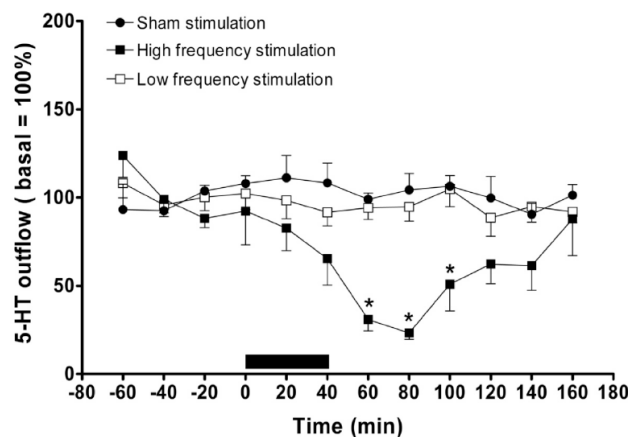

Fig. (2). 5-HT outflow was measured in the dialysate from the caudate putamen of freely moving rats by HPLC with electrochemical detection. Data are mean ± SEM expressed in percent of basal values (=100%). 5-HT outflow in animals treated with HFS (n=9) decreased after stimulation where as in control group (n=15) without HFS and animal treated with LFS (n=16) there was no significant change in 5-HT outflow compared to baseline values.

Neither STN-HFS nor STN-LFS altered the extracellular levels of dopamine (Table **5**). When compared to non-stimulated sham controls, STN-HFS led to a stable and significant increase in the levels of the metabolites of dopamine, DOPAC and HVA, in the caudate putamen. In contrast to the STN-HFS results, STN-LFS caused a significant decrease in the extracellular levels of DOPAC and HVA in the ipsilateral caudate putamen (Table **5**).

No behavioral change was observed before, during or after STN-HFS (data not shown).

4. DISCUSSION

Clinically, deep brain stimulation (DBS), particularly DBS of the subthalamic nucleus (STN) has been linked to psychiatric symptoms as side effects that are associated with serotonergic dysfunction, such as impulsivity, depression and increase of suicidal thoughts and behavior [21, 22]. One of the most important mortality risks following DBS by high frequency stimulation of the STN (STN-HFS) in advanced Parkinson's disease is suicide [8]. In this respect, Temel *et al.* found in animal experiments that STN-HFS inhibited the firing rate of serotonergic neurons in the dorsal raphe nucleus (DRN) and aggravated depressive behavior in rats that were subjected to the forced swim test [12]. Additionally, a review by Miguelez *et al.* gave insights into the modification of different serotonin receptor subclasses in Parkinson's dis-

ease and assumed that in the basal ganglia nuclei, serotonergic innervations come mainly from the DRN [23].

Thus, in this in-vivo neurochemical study the question was raised whether electrical stimulation of the STN modulates the extracellular serotonin levels in the caudate-putamen (CPu) of the rat, and whether these interactions were visible when different stimulation parameters, i.e., high (STN-HFS) or low (STN-LFS) frequencies were applied to the STN. The results described above indicate that when simultaneous microdialysis from the ipsilateral CPu was carried out during STN-HFS, extracellular serotonin (5-HT) levels significantly decreased implying the modification of 5-HT neuronal activity.

As proposed by Feuerstein *et al.* [24], GABAergic axons originating in the globus pallidus externus (GPe), are activated by DBS of the STN to release GABA into the STN. The theory that HFS is affecting the GABAergic fibers projecting to the STN with an increase of the inhibitory GABAergic activity was supported by the observation that the most effective HFS site is located dorsal/dorsomedial to the STN, in the region of the pallidofugal fibers [25, 26]. Therefore, it was assumed that STN-HFS moderates the glutamatergic drive to the globus pallidus internus (GPi) and the substantia nigra pars reticulata (SNr) [24]. Rosenbaum *et al.* showed that placement of the electrode and the amplitude of stimulation maximized the number of STN axons activated and will suppress the transfer of β-oscillations and synchrony from the STN to GPi [27]. These authors described the following connections between STN and serotonergic neurons of the dorsal raphe nucleus in relation to 5-HT-associated side effects of STN-HFS: The STN receives GABAergic projections from the GPe and has glutamatergic projections to the GPi. GABAergic neurons from the GPi project to the thalamus and the lateral habenula (LH) [24]. The LH plays the most important role since it has glutamatergic afferents to the dorsal raphe nuclei (DRN), which via GABAergic interneurons regulate the serotonergic network projecting to the CPu, the prefrontal cortex, and the hippocampus [8-10,12]. Our finding with a significant decrease of 5-HT in the CPu when the STN was stimulated electrically by high frequencies (124 Hz) is, therefore, absolutely in line with the assumptions of Feuerstein *et al.* [24] and also corroborates the electrophysiological results of Temel *et al.* and Tan *et al.* that serotonergic DRN neurons to the CPu are inhibited by STN-HFS [12, 28]. Additionally, findings from Reymann *et al.* give also evidence that the STN plays a role in social behavior and anxogenic like behavior in rodents; thus, these authors proposed that DBS could result in an abnormal brain state where anxiety and social behavior is affected [29]. In this context, Kiser *et al.* conclude that serotonin is positively correlated with sensitivity to social factors and modulates social behavior depending on the nature of social factors [30].

We could, additionally, demonstrate that modification of extracellular 5-HT levels continued well after the stimulation was ceased, but also that the changes were not permanent but reversible over time. We do not have a sufficient explanation for this phenomenon; that STN-HFS leads to an attenuating network oscillation over time is possible but rather speculative. Electrophysiological studies indeed indicated that STN-HFS suppresses oscillatory ß-activities or synchronization in

Table 4. **Mean value ± SEM of 5-HT and HIAA from rat caudate nucleus. Values are given for control animals (sham stimulated) (n=15), LFS-STN (n=16), and HFS-STN (n=9). All values are expressed in percentage and are expressed with respect to baseline=100%. Basal is baseline period, Stim is Stimulation period, Post Stim 1 is Post Stimulation period 1 and Post Stim 2 is Post Stimulation period 2. # represents $p<0.0001$ and * represents $p<0.01$ compared with basal values.**

	5-HT			HIAA		
	HFS	**LFS**	**Control**	**HFS**	**LFS**	**Control**
Basal	100 ± 5.7	100 ± 4.7	100 ± 7.6	100 ± 9.1	100 ± 5.5	100 ± 8.9
Stim	80 ± 11	96 ± 6.7	103 ± 9.5	79 ± 15	92 ± 9.2	91 ± 7.3
Post Stim 1	38 ± 6.5 #	106 ± 6.5	96 ± 6.2	74 ± 12 *	101 ± 12	90 ± 8.9
Post Stim 2	76 ± 11 *	96 ± 7.7	97 ± 8	69 ± 11 *	96 ± 16	96 ± 23

Table 5. **Mean value ± SEM of DA, DOPAC and HVA from rat caudate nucleus. Values are given for control animals (sham stimulated) (n=15), LFS-STN (n=16), and HFS-STN (n=9). All values are expressed in percentage and are expressed with respect to baseline=100%. Basal is baseline period, Stim is Stimulation period, Post Stim 1 is Post Stimulation period 1 and Post Stim 2 is Post Stimulation period 2. # represents $p<0.001$ and * represents $p<0.01$ compared with basal values.**

	Dopamine			Dopac			HVA		
	HFS	**LFS**	**Control**	**HFS**	**LFS**	**Control**	**HFS**	**LFS**	**Control**
Basal	100 ± 9.6	100 ± 4.7	100 ± 15	100 ± 8.9	100 ± 4.6	100 ± 3.9	100 ± 8.2	100 ± 4.5	100 ± 4.8
Stim	115 ± 21	99 ± 7.5	97 ± 21	155 ± 30	88 ± 6.1	94 ± 7.5	167 ± 26 *	87 ± 8.3	114.1 ± 12
Post Stim 1	105 ± 22	106 ± 5.3	103 ± 16	126 ± 22	87 ± 5.3 #	102 ± 8.2	108 ± 20	79 ± 3.8 #	103 ± 7.3
Post Stim	107 ± 20	108 ± 6.3	102 ± 13	136 ± 22	98 ± 15	98 ± 9	121 ± 18	77 ± 3.6 #	94 ± 6.2

parkinsonian patients [31, 32]. It is, however, unclear whether these electrophysiological findings are related to biochemical changes. The prolonged inhibition effect of STN-HFS could probably be attributed to changes in synaptic plasticity at the level of the STN [22]. Therefore, instead of being a simple and direct mechanism, STN-HFS may be responsible for altered function of the neural network between the STN and the DRN.

The effect of lowered 5-HT levels was only limited to high frequency stimulation. When the STN was subjected to low frequency stimulation (30 Hz), we did not see an effect on the levels of 5-HT or HIAA. Hence we can infer that the change in the 5-HT neuronal activity is frequency specific. HIAA levels showed a trend to decrease when STN-HFS was carried out. The decline that was measured in the HIAA levels could be explained to be linked to the decrease in the 5-HT neuronal activity.

When STN-HFS was carried out, significant changes could not be observed in the extracellular levels of dopamine (DA). However, significant increase in its metabolites, 3, 4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) was measured compared to baseline values. These isolated effects on both dopamine metabolites without modification of extracellular dopamine itself after STN stimulation were expected and confirm earlier studies on native and also 6-hydroxydopamine-lesioned rats from Meisner *et al.* [33, 34]. They argued that extracellular dopamine levels do not necessarily reflect concentrations at the synaptic cleft [17]. This assumption was corroborated by findings of Sabol

et al. [35] and Diana *et al.* [36] who could demonstrate only an isolated increase of extracellular striatal DOPAC in rats turning on a treadmill although dopaminergic neurons exhibited increased firing. Thus, enhancement of DOPAC and HVA could give an indirect advice of an activation of the dopaminergic system by electrical STN stimulation. In this context it was suggested by Zetterström *et al.* [37] and also Imperio & Di Chiara [38] that extracellular DOPAC and HVA levels may indicate intraneuronal dopamine synthesis. Since electrical high frequency stimulation indeed led to an increase in firing activity of nigral DA neurons [39], it should be stated that extracellular dopamine levels measured from micro dialysate samples from the CPu do definitively not reflect dopaminergic activity of the nigrostriatal pathway. According to Feuerstein *et al.* [24], STN-HFS reinforce GABAergic inhibition on glutamatergic neurons of the STN. This leads to a decline of glutamatergic activity on substantia nigra pars reticulata (SNr). Since dopaminergic neurons in the substantia nigra pars compacta (SNc) are inhibited by GABAergic afferents from the SNr [40] and STN-HFS is presumed to indirectly inhibit these GABAergic neurons [39], high frequency stimulation induce SNc disinhibition with an increase of dopaminergic activity and metabolism.

When low frequency stimulation (STN-LFS) was carried out, there was no effect on dopamine levels as found in STN-HFS, but a significant drop in extracellular levels of both DOPAC and HVA. The decrease did not recover over the time period of the experiments that were conducted up to 120 minutes. This finding led us to suggest that the basal ganglia network is also susceptible to STN-LFS [41-43],

although another biochemical mechanism should be discussed as in the case of STN-HFS. It has been reported that STN-LFS (< 30 Hz) in humans can impair movement with slow distal upper limb movements and worsening of akinesia in Parkinson's patients [26,41], which could be explained by an activation of the glutamatergic efferents from the STN.

Taken together, our results provide evidence that electrical high frequency stimulation of the subthalamic nucleus causes indeed a significant decrease in the 5-HT neuronal activity in the caudate-putamen of the rat probably as a result of inhibition of serotonergic neurons arising from the DRN. This effect was found to be frequency dependent and was only seen with high frequency stimulation (124 Hz). The dopaminergic system was also affected by STN stimulation with modulation of extracellular DOPAC and HVA levels but without biochemical changes of extracellular dopamine.

LIST OF ABBREVIATIONS

HFS	=	High Frequency Stimulation
LFS	=	Low Frequency Stimulation
STN	=	Subthalamic nucleus
5HT	=	5-Hydroxytryptamine
HIAA	=	5-Hydroxyindolic acid
DA	=	Dopamine
DOPAC	=	3, 4-Dihydroxyphenylacetic acid
HVA	=	Homovanillic acid
CPu	=	Caudate putamen
LFS	=	Low frequency Stimulation
L-Dopa	=	Levo-Dopa
DRN	=	Dorsal raphe nucleus
aCSF	=	Artificial cerebrospinal fluid
HPLC-ED	=	High Pressure Liquid Chromatography with Electrochemical Detection
DBS	=	Deep Brain Stimulation
GABA	=	Gamma-aminobutyric acid
GPe	=	Globus pallidus externus
GPi	=	Globus Pallidus Internus
SNr	=	Substantia nigra pars reticulata
LH	=	Lateral habenula
SNc	=	Substantia nigra pars compacta

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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PATIENT'S CONSENT

Declared none.

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